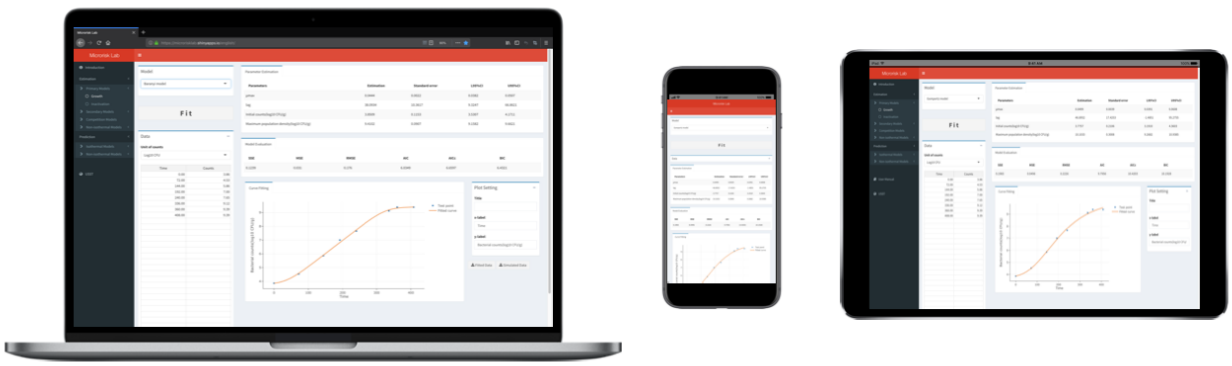
Microrisk Lab

User Manual

Revised at January 2018

Version 1.0



Disclaimer and Support

Disclaimer

Microrisk Lab and this manual provides NO WARRANTY. This tool is free to use but only for research purposes. It is not permitted to include Microrisk Lab in any other application. We would very appreciate acknowledgement if the tool is used.

Feedback

If you have any suggestion or for technical questions for Microrisk Lab, please contact the developer and maintainer Yangtai Liu (usstlyt@163.com), Your comments are highly appreciated.

Document Revisions

Date	Version	Document Changes
01/09/2017	Beta 0.1	Initial Draft
01/01/2018	1.0	Draft for Updated version

Contents

1 List of symbols

$Y(t)$, Y_0, Y_{max}	the natural logarithm of real-time, initial, and maximum bacterial counts (ln CFU/g).
$y(t)$, y_0, y_{max}	the 10-base logarithm of real-time, initial, and maximum bacterial counts (log10 CFU/g).
y_{res}	the 10-base logarithm of the residual bacterial counts (log10 CFU/g).
μ_{max}	the maximum and optimal specific growth rate.
k_{max}	the maximum specific inactivation rate.
D	the decimal reduction time.
D_{ref}	the referenced decimal reduction time.
t_{lag}	the time of lag in growth.
S_l	the time of shoulder (or before inactivation) in inactivation.
t_{max}	the time arriving stationary phase in growth.
S_t	the time arriving tail (or stationary phase) in inactivation.
T_{min}, T_{opt}, T_{max}	the minimum, optimal, and maximum growth temperature (°C).
T_{ref}	the referenced inactivation temperature (°C).
pH_{min}, pH_{opt}, pH_{max}	the minimum, optimal, and maximum growth pH.
aw_{min}, aw_{opt}, aw_{max}	the minimum, optimal, and maximum growth water activity.
q_0	the initial physiological state of the inoculum in the Baranyi model.
δ, p	the coefficients in the Weibull model.
a, b	the coefficients in the square-root model.
A, m	the coefficients in the dynamic Huang model.

2 Unit

The unit of bacterial count and time related variables can be defined by the user. The unit of predicted counting outputs will be transfer into 10-base logarithm. Note that the unit of the specific (growth/ inactivation) rate is a natural logarithm combined with unit of time, for example, ln CFU/g/h or ln CFU/g/min.

3 Programing basics

Microrisk Lab is developed by the open-source language R (version 3.5.1 for Mac OS X; http://ww.r-project.org). All users are free to access and use this tool through the browser of any internet-connected device by the following link:

http://microrisklab.shinyapps.io/english

The operation of this Microrisk Lab must depend on the certain developed R packages, which were listed in Tab.1. All the required packages have been hosted and deployed in the Shinyapps.io sever (https://www.shinyapps.io).

Tab.1 Imported R packages in Microrisk Lab

Package name	Version	Reference	Purpose
ggplot2	2.2.1	H. Wickham	to generate visualized plots for output
mc2d	0.1-18		to generate certain distribution for output
Metrics	0.1.3		to calculate statistical indicators for output
plotly	4.7.1		to generate interactive plots for output
rhandsontable	0.3.6		to build interactive table for input
shiny	1.0.5		to establish and upload the shiny app
shinyalert	1.0		to pop the error alert for input and output
shinydashboard	0.7.0		to build the interactive interface
shinyWidgets	0.4.2		to build the interactive interface
stats	3.4.3		to realize the regression analysis

Microrisk Lab can be also used on computers without internet connection when installed locally. In this case, please contact the developer.

4 Functions included in Microrisk Lab

Microrisk Lab includes the following functions:

- Kinetic analysis of microbial isothermal growth
- Kinetic analysis of microbial non-isothermal growth
- Kinetic analysis of microbial isothermal inactivation
- Kinetic analysis of microbial non-isothermal inactivation
- Kinetic analysis of two-flora isothermal competition growth
- Secondary modeling of specific growth rate vs. temperature, pH and Aw.
- Deterministic/ Stochastic simulation for microbial isothermal growth
- Deterministic simulation for microbial isothermal growth
- Deterministic/ Stochastic simulation for microbial isothermal inactivation
- Deterministic simulation for microbial isothermal inactivation
- Output interactive plots of fitted and predicted curves.
- Output estimated results (estimates, standard error, and 95% confidential intervals) and multiple statistical indicators (RSS, MSE, RMSE, AIC, AICc, BIC) with respect to the experimental data in the ‘Estimation’ module.
- Output simulated bacterial counts or the distribution of the specific rate and final bacterial counts in the ‘Simulation’ module.
- Output correlation analysis between model parameters and simulated bacterial counts in the stochastic simulation.

5 Layout of Microrisk Lab

Fig.1 shows the page structure when loading in the Microrisk Lab via the browser in different devices. Users may switch the target question by the main menu in the left side. In the setting panel, user can input the experiment data and choose the model in here. The result panel will provide the estimated (predicted) values, statistical results, and interactive plots according to the setting.

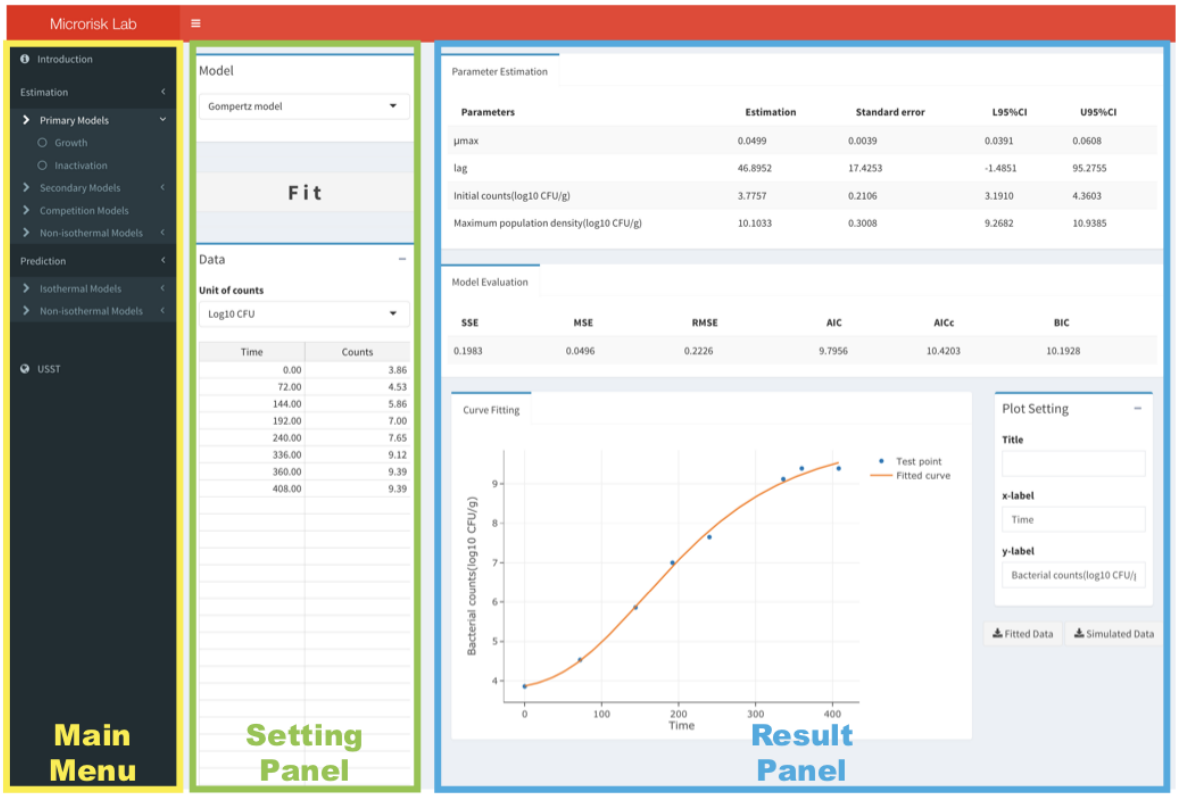


Fig.1 Typical layout of Microrisk Lab.

6 Estimation module of Microrisk Lab

The estimation module allows to solve multiple inverse problems in predictive microbiology, including ① isothermal growth fitting, ② isothermal inactivation fitting, ③④⑤ secondary model fitting, ⑥ two flora competition growth fitting, ⑦ non-isothermal growth fitting, and ⑧ non-isothermal inactivation fitting (Fig.2).

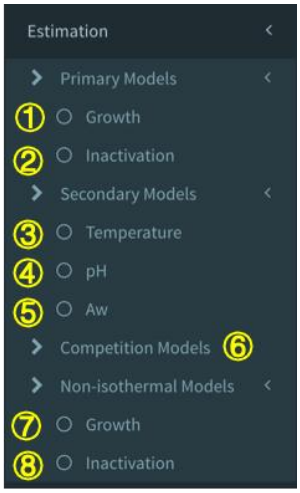


Fig.2 Different sections of model fitting in the estimation module.

Practical example Ⅰ- Isothermal growth fitting

(1) Choose ① the 'Growth' in the section of the 'Primary Models', and the setting panel of isothermal growth model will show up (Fig.3).

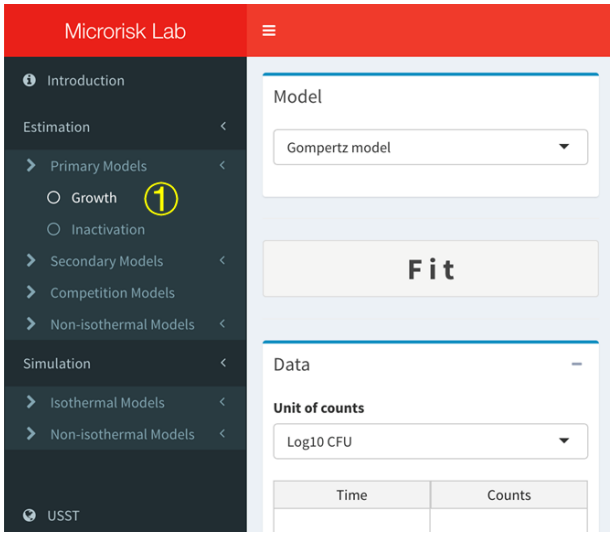


Fig.3 Layout of a section of the 'Estimation' module.

(2) The experimental data can be ① directly typed (or ② copied from other table files) in the 'Data' box. Specifically, ③ the unit of bacterial counts should be confirmed by the user. If the inputted observations are more than 30, please ④ right click the mouse or ⑤ drag the last column to add additional columns. Here, a group of *Listera monocytogenes*/ *innocua* growth in tryptose phosphate broth (TPB) obtained from the ComBase database (www.combase.cc, ComBase ID: LM127_11) was used as the test dataset (Fig.4).

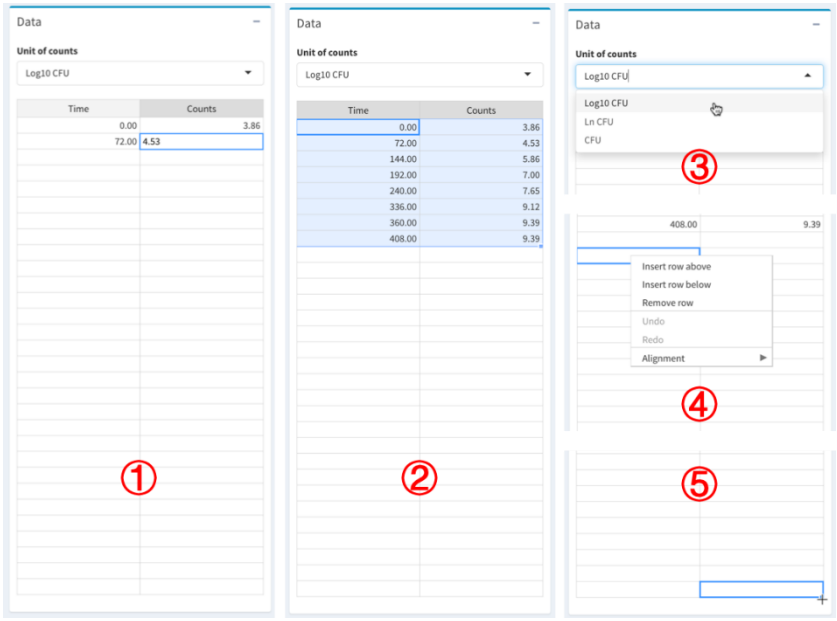


Fig.4 Boxes for the data input and unit selection.

(3) After entering the data for model fitting, the growth model can be selected in the 'Model' list (Fig.5).

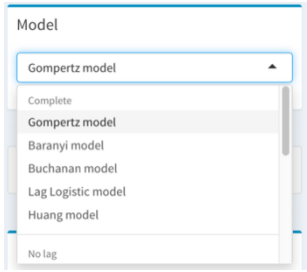


Fig.5 Box for model selection

(4) Click ① the 'Fitting' button. After a necessary loading time, if the regression can be solved successfully, the ② estimated result, ③ evaluated result, and ④ interactive plot of the observation and fitted curve will show in the result panel (Fig.6A). Otherwise, a popup message

will appear for the regression warning, which means that the non-linear regression is failed (Fig.6B). In this case, please check the unit of bacterial counts and the model selection.

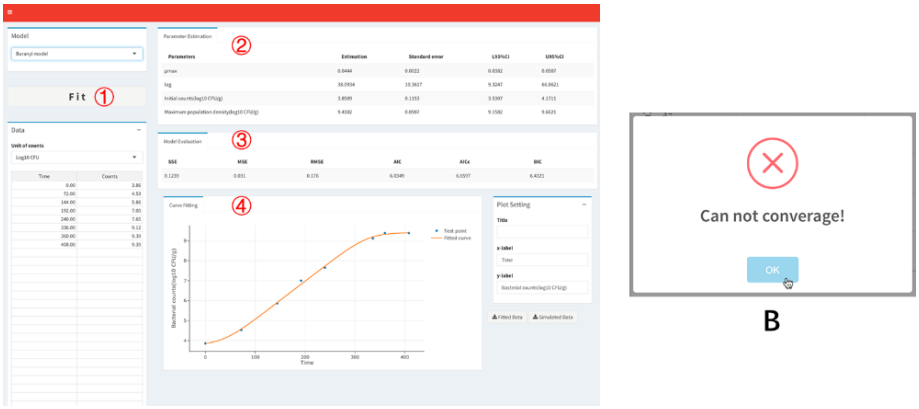


Fig.6 Layout of the interface after model fitting.

(5) The observed and predicted value can be viewed on ① the interactive plot. The observed data or fitting curve can be omitted from the plot by clicking ② the legend. Meanwhile, it is easy to edit the axis detail (③ the range and ④ title) of the interactive plot in real-time by ⑤ the box of 'Plot Setting'. After all, the plot is adjustable and downloadable by using ⑥ the 'Plotly toolbox'. Meanwhile, ⑦ the fitted and simulated data can be saved as the '.csv' file for comparison and further model development (Fig.7).

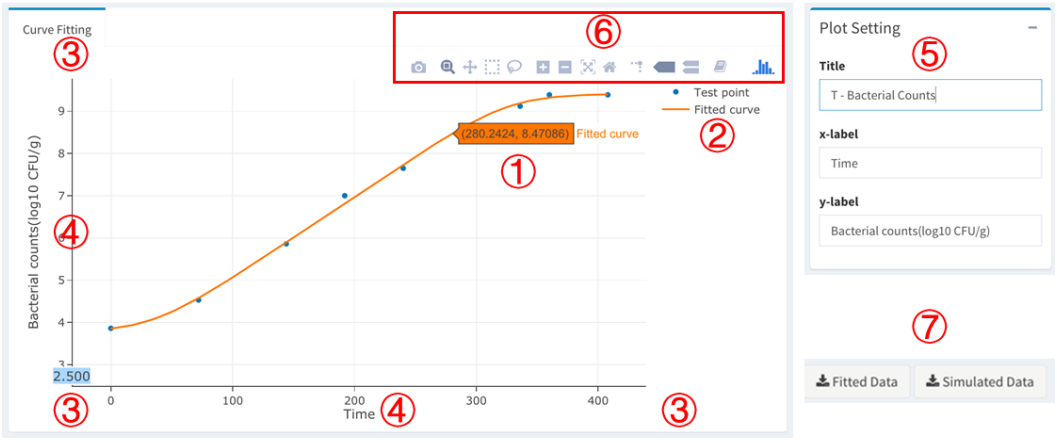


Fig.7 The interactive plot and the editorial box.

(6) Note that, in the section of 'Non-isothermal Models', the additional time-temperature profile is needed to be imported for dynamic fitting.

7 Simulation module of Microrisk Lab

The simulation module allows to solve the ①② isothermal and ③④ non-isothermal forward problem in predictive microbiology (Fig.8). There are no limitations in the condition setting. Users may simulate the bacterial growth or inactivation with the prior knowledge on the kinetic parameter and growth/ death boundary. Moreover, both deterministic and stochastic models are provided in the isothermal simulation.

Fig.8 Different sections of model fitting in the simulation module.

Practical example Ⅱ- Stochastic growth simulation

(1) The condition setting of the growth simulation is adopted from the stochastic growth of *Salmonella* Typhimurium individual cells researched by Koutsoumanis and Lianou (2013). Tab.2 lists the setting for simulation. The Buchanan model is chosen as the growth model for individual cells. A 10,000 times iteration was realized based on the Monte-Carlo simulation method.

Tab.2 Stochastic growth simulation settings for Microrisk Lab

Parameters	**Microrisk Lab**	
	Distribution	Normal
y_0 ($\log_{10}$ CFU/g)	Mean	0
	Standard deviation	0
	Distribution	Normal
y_{max} ($\log_{10}$ CFU/g)	Mean	8
	Standard deviation	0
	Distribution	LogNormal
t_{lag}	Mean	3.355
	Standard deviation	0.896
	Shift	-1.628
	Distribution	Logistic
μ_{max}	Mean	0.754
	Standard deviation	0.024
	Distribution	Uniform
t	Maximum	0
	Minimum	8
Model	Buchanan model	
Iteration times	10,000	

(2) Choose ① the ‘Growth’ section of the ‘Isothermal Models’ in the ‘Simulation’ module, and ② choose ‘Stochastic’ model type in the setting panel (Fig.9). Then set the ③ ‘Iteration time’ and ④ ‘Model’ to ‘10,000’ and ‘Buchanan model’, respectively.

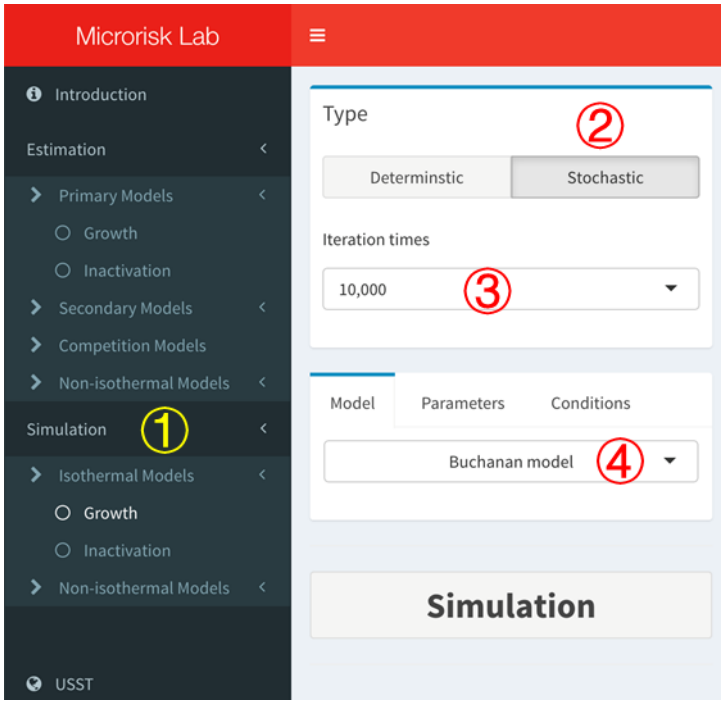


Fig.9 Layout of a section of the 'Simulation' module.

(3) Switch to the ① 'Parameters' tab to determine the setting of the (distribution of) ② y_{max}, ③ t_{lag}, and ④ μ_{max} according to Tab.2 (Fig.10).

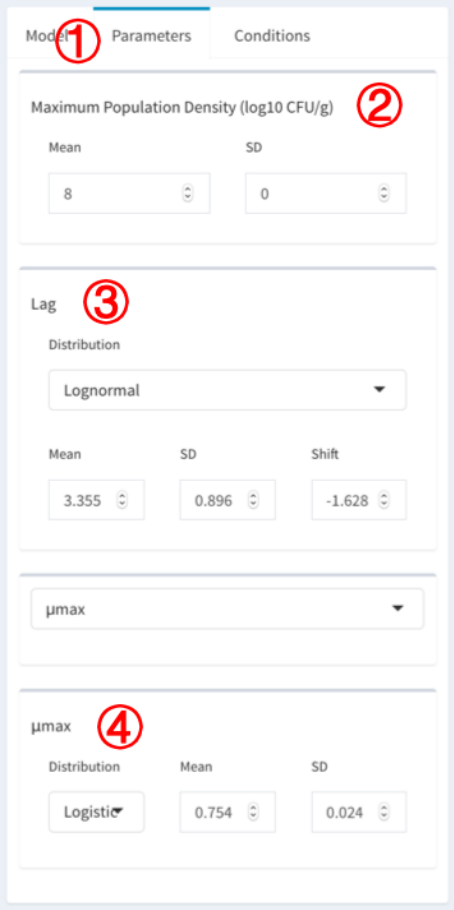


Fig.10 Box for kinetic parameter setting.

(4) Switch to the ① 'Conditions' tab to determine the setting of the (distribution of) ② y_0, ③ t according to Tab.2 (Fig.11).

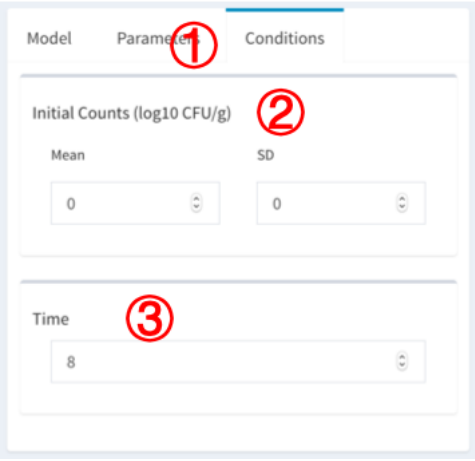


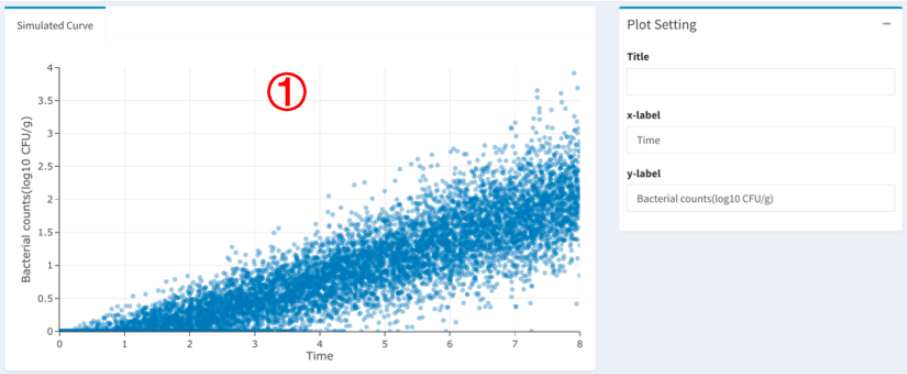
Fig.11 Box for condition setting.

(5) Click the ① 'Simulation' button. After a necessary loading time, if no contradiction in the setting, the ② simulated curve/point and ③ predicted result will show in the result panel

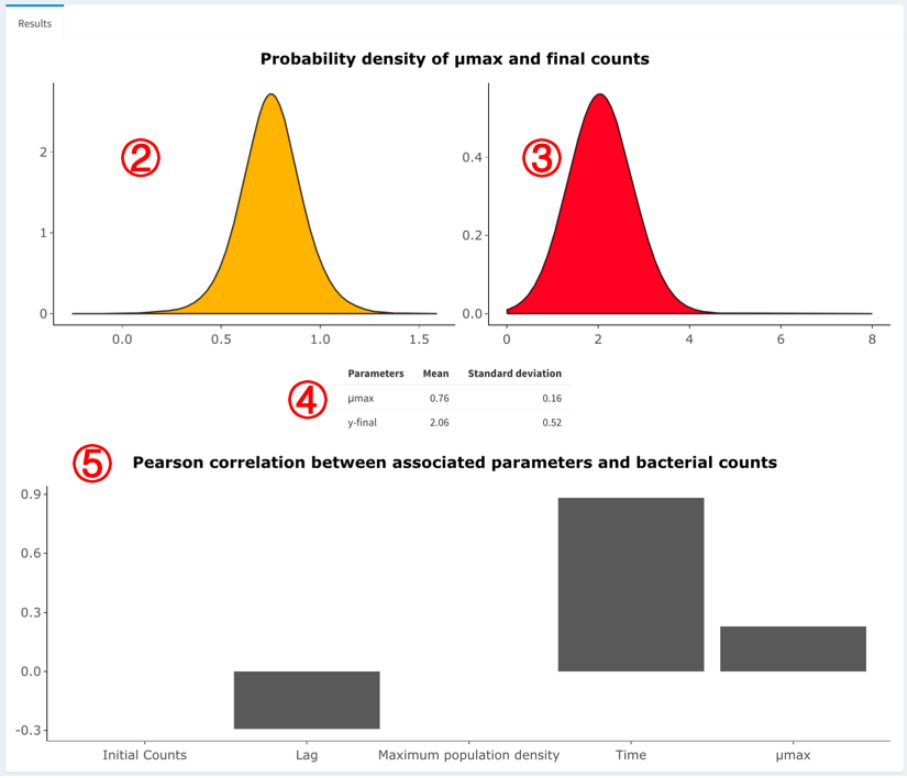
(Fig.12A). Otherwise, different popup messages will appear for the simulation warning (Fig.12B-D). In these cases, please check the setting of kinetic parameters and the condition of simulated environment.

Fig.12 Layout of the interface after simulation.

(6) The stochastic growth simulation can be viewed on ① the interactive plot, which is also adjustable and downloadable (Fig.13A). The distribution of ② the estimated μ_{max} and ③ final bacterial concentration (y_{final}) , as well as ④ the estimated mean value and standard deviation will be presented and listed (Fig.13B). The sensitivity analysis on model parameters is realized by calculating the Pearson correlation between different factors and the bacterial counts. Here, according to ⑤ the correlation plot, the duration of growth time is the most sensitive parameter for the bacterial counts during the stochastic growth of *S.* Typhimurium single cell (Fig.13B).



A



B

Fig.13 The result of stochastic simulation.

(7) Note that, in the section of 'Non-isothermal Models' of the simulation module, only deterministic model was provided in this version.

8 Predictive models integrated in Microrisk Lab

Microrisk Lab consists of 11 isothermal growth models (Tab.2), 9 inactivation models (Tab.3),10 secondary models (Tab.4), 2 competition growth models (Tab.5), and 4 non-isothermal models (Tab.6) for estimation or simulation works.

Tab.2. Isothermal growth models included in Microrisk Lab

Name	Formula
Complete model	
Gompertz model [1]	$Y(t) = Y_0 + (Y_{max} - Y_0)exp\left\{-\exp\left[\frac{2.71\mu_{max}(t_{lag}-t)}{Y_{max}-Y_0} + 1\right]\right\}$
Baranyi model [2]	$\begin{cases} Y(t) = Y_0 + A(t) - \ln\left[1 + \frac{\exp(\mu_{max}A(t))-1}{\exp(Y_{max}-Y_0)}\right] \\ A(t) = \mu_{max}t + \ln[\exp(-\mu_{max}t) + \exp(-h_0) - \exp(-\mu_{max}t - h_0)] \\ h_0 = \mu_{max}t_{lag} \end{cases}$
Buchanan model [3]	$\begin{cases} y(t) = y_0, \ t < t_{lag} \\ y(t) = y_0 + \frac{\mu_{max}}{\ln 10}(t - t_{lag}), \ t_{lag} \le t < t_{max} \\ y(t) = y_{max}, \ \ t \ge t_{max} \end{cases}$
Lag logistic model [4]	$\begin{cases} Y(t) = Y_0, \ t < t_{lag} \\ Y(t) = Y_{max} - \ln\{1 + [\exp(Y_{max} - Y_0) - 1]\exp[-\mu_{max}(t - t_{lag})]\}, \ t \ge t_{lag} \end{cases}$
Huang model [5]	$\begin{cases} Y(t) = Y_0 + Y_{max} - \ln\{\exp(Y_0) + [\exp(Y_{max}) - \exp(Y_0)]\exp(-\mu_{max}B(t))\} \\ B(t) = t + \frac{1}{\alpha}\ln\frac{1+\exp[-\alpha(t-t_{lag})]}{1-\exp(\alpha t_{lag})} \end{cases}$
No lag model	
Logistic model [6]	$Y(t) = Y_0 + Y_{max} - \ln\{\exp(Y_0) + [\exp(Y_{max}) - \exp(Y_0)]\exp(-\mu_{max}t)\}$
Buchanan model [7]	$\begin{cases} y(t) = y_0 + \frac{\mu_{max}}{\ln 10}t, \ t < t_{max} \\ y(t) = y_{max}, \ t \ge t_{max} \end{cases}$
Reduced model	
Baranyi model [8]	$\begin{cases} Y(t) = Y_0 + \mu_{max}t + \ln[\exp(-\mu_{max}t) + \exp(-h_0) - \exp(-\mu_{max}t - h_0)] \\ h_0 = \mu_{max}t_{lag} \end{cases}$
Buchnan model [9]	$\begin{cases} y(t) = y_0, \ t < t_{lag} \\ y(t) = y_0 + \frac{\mu_{max}}{\ln 10}(t - t_{lag}), \ t \ge t_{lag} \end{cases}$
Huang model [10]	$Y(t) = Y_0 + \mu_{max}t + \frac{1}{\alpha}\mu_{max}\ln\frac{1+\exp[-\alpha(t-t_{lag})]}{1-\exp(\alpha t_{lag})}$
Linear model	$Y(t) = Y_0 + \mu_{max}t$

[1]Zwietering et al., 1990; [2/8] Baranyi and Roberts, 1995; [3/7/9] Buchanan et al., 1997; [4] Rosso et al., 1996; [5/6/10] Huang, 2008.

Tab.3. Isothermal inactivation models included in Microrisk Lab

Name	Formula
Completed Geeraerd model [1]	$y(t) = y_{res} + \log_{10}\left[\frac{(10^{y_0 - y_{res}} - 1)\exp(k_{max}S_l)}{\exp(k_{max}t) + \exp(k_{max}S_l) - 1} + 1\right]$
Three-phase model [2]	$\begin{cases} y(t) = y_0, \ t < S_l \\ y(t) = y_0 + \frac{k_{max}}{\ln 10}(t - S_l), \ S_l \le t < S_t \\ y(t) = y_{res}, \quad t \ge S_t \end{cases}$
Weibull-tail model [3]	$y(t) = y_{res} + \log_{10}\left[(10^{y_0 - y_{res}} - 1)\, 10^{-\left(\frac{t}{\delta}\right)^p} + 1\right]$
No shoulder Geeraerd model [4]	$y(t) = y_{res} + \log_{10}\{(10^{y_0 - y_{res}} - 1)\exp(k_{max}t) + 1\}$
No shoulder two-phase model [5]	$\begin{cases} y(t) = y_0 + \frac{k_{max}}{\ln 10}t, \ t < S_t \\ y(t) = y_{res}, \quad t \ge S_t \end{cases}$
No tail Geeraerd model [6]	$y(t) = y_0 + \frac{k_{max}t}{\ln 10} + \log_{10}\left\{\frac{\exp(k_{max}S_l)}{1 + [\exp(k_{max}S_l) - 1]\exp(k_{max}t)}\right\}$
No tail two-phase model [7]	$\begin{cases} y(t) = y_0, \ t < S_l \\ y(t) = y_0 + \frac{k_{max}}{\ln 10}(t - S_l), \quad t \ge S_l \end{cases}$
Weibull model [8]	$y(t) = y_0 - \left(\frac{t}{\delta}\right)^p$
Bigelow model [9]	$y(t) = y_0 - \frac{t}{D}$

[1/ 4/ 6] Geeraerd et al., 2000; [2/ 5/ 7] Buchanan and Golden, 1995; [3] Albert and Mafart, 2005; [8] van Boekel, 2002; [9] Bigelow, 1921.

Tab.4. Secondary models for μ_{max} included in Microrisk Lab

Name	Formula
Temperature models	
Suboptimal square-root model [1]	$\mu_{max} = [a(T - T_{min})]^2$
Full square-root model [2]	$\mu_{max} = \langle a(T - T_{min})\{1 - \exp[b(T - T_{max})]\}\rangle^2$
Suboptimal Huang square-root model [3]	$\mu_{max} = [a(T - T_{min})^{0.75}]^2$
Full Huang square-root model [4]	$\mu_{max} = \langle a(T - T_{min})^{0.75}\{1 - \exp[b(T - T_{max})]\}\rangle^2$
Cardinal parameter model [5]	$\mu_{max} = \frac{\mu_{opt}(T-T_{max})(T-T_{min})^2}{[(T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)](T_{opt}-T_{min})}$
pH models	
Cardinal 3-parameter model [6]	$\mu_{max} = \frac{\mu_{opt}(pH-pH_{min})[pH-(2pH_{opt}-pH_{min})]}{(pH-pH_{min})[pH-(2pH_{opt}-pH_{min})]-(pH-pH_{opt})^2}$
Cardinal 4-parameter model [7]	$\mu_{max} = \frac{\mu_{opt}(pH - pH_{min})(pH - pH_{max})}{(pH - pH_{min})(pH - pH_{max}) - (pH - pH_{opt})^2}$
Quasi-mechanistic model [8]	$\mu_{max} = \mu_{opt}(1 - 10^{pH_{min}-pH})$
Water activity models	
Cardinal 2-parameter model [9]	$\mu_{max} = \frac{\mu_{opt}(aw-aw_{min})^2}{(1-aw_{min})^2}$
Cardinal 3-parameter model [10]	$\mu_{max} = \frac{\mu_{opt}(aw-1)(aw-aw_{min})^2}{(aw_{opt}-aw_{min})[(aw_{opt}-aw_{min})(aw-aw_{opt})-(aw_{opt}-1)(aw_{opt}+aw_{min}-2aw)]}$

[1/2] Ratkowsky et al., 1983; [3/4] Huang and Hwang, 2011; [5] Rosso et al, 1993; [6/7] Rosso et al, 1995; [8] Presser et al. 1997; [9/10] Rosso and Robinson, 2001

Tab.5. Two flora competition growth models included in Microrisk Lab

Name	Formula
Jameson - No lag Buchanan model [1]	$\begin{cases} y_1(t) = \begin{cases} y_1 + \frac{\mu_{max1}}{\ln 10} t, & t < t_{max} \\ y_1 + \frac{\mu_{max1}}{\ln 10} t_{max}, & t \geq t_{max} \end{cases} \\ y_2(t) = \begin{cases} y_2 + \frac{\mu_{max2}}{\ln 10} t, & t < t_{max} \\ y_2 + \frac{\mu_{max2}}{\ln 10} t_{max}, & t \geq t_{max} \end{cases} \end{cases}$
Jameson - Buchanan model [2]	$\begin{cases} y_1(t) = \begin{cases} y_1, & t < t_{lag1} \\ y_1 + \frac{\mu_{max1}}{\ln 10}(t - t_{lag1}), & t_{lag1} \leq t < t_{max} \\ y_1 + \frac{\mu_{max1}}{\ln 10}(t_{max} - t_{lag1}), & t \geq t_{max} \end{cases} \\ y_2(t) = \begin{cases} y_2, & t < t_{lag2} \\ y_2 + \frac{\mu_{max2}}{\ln 10}(t - t_{lag2}), & t_{lag2} \leq t < t_{max} \\ y_2 + \frac{\mu_{max2}}{\ln 10}(t_{max} - t_{lag2}), & t \geq t_{max} \end{cases} \end{cases}$

[*] The inferior number 1 or 2 in competition growth models represent the flora type.

[1/2] Vimont et al., 2006

Tab.6. Non-isothermal models included in Microrisk Lab

Name	Formula
Non-isothermal growth models	
Baranyi - Cardinal parameter model [1]	$\begin{cases} \frac{dY(t)}{dt} = \mu_{max}\left[\frac{1}{1+\exp(-Q(t))}\right][1 - \exp(Y(t) - Y_{max})] \\ \frac{dQ(t)}{dt} = \mu_{max} \\ Q(t) = \ln\frac{q(t)}{1-q(t)} \\ \mu_{max} = \frac{\mu_{opt}(T-T_{max})(T-T_{min})^2}{[(T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)](T_{opt}-T_{min})} \end{cases}$
Huang - Cardinal parameter model [2/3]	$\begin{cases} \frac{dY(t)}{dt} = \mu_{max}\left[\frac{1}{1+\exp(-4(t-t_{lag}))}\right][1 - \exp(Y(t) - Y_{max})] \\ t_{lag} = \frac{\exp(A)}{{\mu_{max}}^m} \\ \mu_{max} = \frac{\mu_{opt}(T-T_{max})(T-T_{min})^2}{[(T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)](T_{opt}-T_{min})} \end{cases}$
Non-isothermal inactivation model	
Dynamic Bigelow model [5]	$\frac{dy}{dt} = -\frac{1}{D_{ref}} 10^{\frac{T-T_{ref}}{z}}$

[1/2/3] Huang, 2017; [5] Van Impe et al., 1992.

9 Statistical indicators in Microrisk Lab

To evaluate and compare the goodness of fit, the statistical indicator of residual sum of squares (RSS, Eq.1), mean square error (MSE, Eq.2), root mean square error (RMSE, Eq.3), regular Akaike information criterion (AIC, Eq.4, Akaike, 1974), modified AIC (AICc, Eq.5, van Boekel et al., 2007; Huang, 2014) and Bayesian information criterions (BIC, Eq.6, Schwarz, 1978). are provided such in the 'Model Evaluation' tab.

$$\mathrm{RSS} = \sum_{i=1}^{n}(y_i - \hat{y}_i)^2 \qquad \text{Eq.1}$$

$$\mathrm{MSE} = \frac{\mathrm{RSS}}{n-k} \qquad \text{Eq.2}$$

$$\mathrm{RMSE} = \sqrt{\mathrm{MSE}} \qquad \text{Eq.3}$$

$$\mathrm{AIC} = n \ln\left(\frac{\mathrm{RSS}}{n}\right) + 2(k+1) \qquad \text{Eq.4}$$

$$\mathrm{AIC_c} = n \ln\left(\frac{\mathrm{RSS}}{n}\right) + 2(k+1) + \frac{2(k+1)(k+2)}{n-k-2} \qquad \text{Eq.5}$$

$$\mathrm{BIC} = n \ln\left(\frac{\mathrm{RSS}}{n}\right) + k \ln(n) \qquad \text{Eq.6}$$

Where y_i is the i th value of the observation; $\hat{y}_i$ is the i th value of the prediction; k is the number of parameters; n is the number of sample data; L is the maximum value of the likelihood function for the model.

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